Structural Biology of Antibiotic Resistance

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ANTIBIOTICS; DRUG RESISTANCE; BETA-LACTAMASES

My primary research interest is to investigate the structure:function relationships in bla enzymes that cause drug-resistance. This proposal seeks to test the following hypotheses regarding resistance to clavulanic acid and sulbactam among Enterobacteriaciae that contain mutants of the common beta-lactamase enzyme, SHV: first, that resistance results from subtle structural changes in the enzyme active site that influence initial attractive interaction and binding of the inhibitor molecule, and secondly, that resistance has not developed toward tazobactam, an inhibitor compound that is structurally similar to sulbactam, because of differences in the chemistry of ring opening versus differences in the initial binding interactions of this compound when compared to related inhibitors. These hypotheses will be tested by obtaining the non-resonance Raman difference (NRRD) spectra of a series of mutant enzymes whose binding of inhibitors will be representative of the postulated series of enzyme: inhibitor interactions that take place that lead to clinically relevant inhibition of the enzymes. These include a catalytically deficient enzyme, S70A, whose binding of the inhibitor will represent the non-bonded Michaelis interaction of the inhibitor and enzyme; the deacylation deficient El66A mutant of SHV which will trap the most stable acyl enzyme intermediate; the wild type enzyme; and finally the clavulanic acid and sulbactam resistant mutants, M691, -L, and V of SHV. Raman methods have never been applied to the study of bla enzymes but have been used successfully to study related serine proteases. This method allows the direct comparison of solution phase and crystal phase enzyme: inhibitor structures. It is believed that this method will provide structural data of higher resolution than X- crystallography and this is important because X-ray methods have not elucidated the structural basis for resistance in these enzymes to date. Such detailed information regarding the important attractive interactions between bla inhibitors and their target enzymes will lead to new beta-lactamase inhibitors of clinical relevance in fighting infections with resistant Gram-negative organisms.